DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: FEB 06 2004

From: Interdisciplinary Scientist/Botanist, Division of Dietary Supplement Programs, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-810

Subject: 75-Day Pre-market Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

Subject of the Notification: Coriolus versicolor and melatonin

Firm: BioAge Pharmaceuticals, Inc.

Date Received by FDA: 7/17/03

90-Day Date: 10/15/03

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day pre-market notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Tanya L. Jackson

Tanya Jackson, Interdisciplinary Scientist, HFD 810

95S-0316

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration
College Park, Maryland 20740

OCT - 3 2003

Mr. William W. Lee
BioAge Pharmaceuticals, Inc.
9924 Mesa Rim Road
San Diego, California 92121

Dear Mr. Lee:

This is to inform you that the notification, dated July 01, 2003, you submitted pursuant to 21 U.S.C. 350b(a)(2)(section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)) was received by the Food and Drug Administration (FDA) on July 17, 2003. Your notification concerns a proteoglycan extract of Coriolus versicolor and melatonin which you call MuneGard.

According to the notification, the dietary supplement called MuneGard will be sold in capsules which contain 500 mg proteoglycan extract of Coriolus versicolor and 10 mg melatonin. You recommend 2 capsules/day (1 g proteoglycan extract of Coriolus versicolor and 20 mg melatonin) by mouth prior to bedtime.

Under 21 U.S.C. 350b(a)(2), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

According to the notification, the MuneGard supplement consists of a proteoglycan extract from Coriolus versicolor and melatonin. However, the notification did not identify the new dietary ingredient(s) in the component dietary supplement called MuneGard.

The notification states that there are a series of polysaccharopeptide (PSP) products depending on "different stages of extraction". However, the notification did not identify the source and identity of the polysaccharopeptide (PSP) in the MuneGard supplement. Additionally, the notification refers to similar substance called polysaccharide kuresa (PSK) and states that "conclusions from studies can be generally applicable to both PSP and PSK". However, PSK is extracted from a a different strain of Coriolus versicolor and contains six times more protein than PSP and differs from PSP in amino acid and and monosaccharide content.

The notification contains a number of published articles dealing with the use of the mushroom proteoglycans PSK and PSP in patients with various cancers. There is no information provided that relates these two materials to the MuneGard preparation that is the subject of the notification. Therefore, your submission provides no information that the test substances used in the referenced studies are qualitatively or quantitatively similar to your ingredient or how these studies are relevant to evaluating the safe use of your ingredient under the recommended conditions of use.

For the reasons discussed above, the information in your submission does not provide an adequate basis to conclude that the MuneGard product, when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Your notification will be kept confidential for 90 days after the filing date of July 17, 2003. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

If you have any questions concerning this matter, please contact Victoria Lutwak at (301) 436-2375.

Sincerely yours,

Susan J. Walker, M.D.
Division Director
Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition

BioAge Pharmaceuticals, Inc.
9924 Mesa Rim Road, San Diego, CA 92121 USA

July 1, 2003

Division of Programs and Enforcement Policy
Office of Special Nutritionals (HFS-456), CFSAN
U.S. Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

RE: 75-day Premarket Notification for New Dietary Ingredient

Dear Sir/Madam:

Pursuant to the requirements of Section 413 (a)(2) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 350B), BioAge Pharmaceuticals, Inc. wishes to notify US FDA that it will market a branded dietary supplement, [redacted], for the interstate commerce 75 days after this notice. Accordingly, two copies of this notification are submitted for your reference.

MuneGard consists of two active ingredients, proteoglycan extract of *Coriolus versicolor* and melatonin. The new dietary ingredient will be sold in capsules and each capsule contains 500mg proteoglycan extract of *Coriolus versicolor* and 10mg melatonin. The recommended use is taking 2 capsules orally (1g proteoglycan extract of *Coriolus versicolor* and 20mg melatonin) each day prior to bedtime.

Both ingredients have been available and are still widely marketed as dietary supplement in the US since 1994.

We conclude that both ingredients are safe based on the following considerations:

a. High LD50 in animal studies. Published reports show that both ingredients have very high LD50 in rodents, over 20g/kg for proteoglycan extract of *Coriolus versicolor* and more than 1g/kg for melatonin, respectively. Compounds with LD50 in this range are generally considered safe.

b. Neither melatonin nor proteoglycan extract of *Coriolus versicolor* induces mutation or genetic instability.

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Phone (858)457-0899 Fax (858)457-0942

c. Neither agent interferes with drug metabolism pathway and no adverse drug interactions are expected.

d. Extensive clinical studies of melatonin as well as proteoglycan of *Coriolus versicolor* demonstrate clinical safety of both agents. 300mg or more melatonin was administrated to patients without significant adverse reactions. For proteoglycan extract of *Coriolus versicolor*, 3 g per day was given to patients for several years without any toxicity.

e. Both agents have been used extensively in clinical practice and show high safety profile.

MuneGard, a new dietary supplement containing both ingredients, is reasonably expected to be safe under the recommended directions for use. Here, we submit selected and indexed references with a summary for each reference and original copy attached to demonstrate the safety of both ingredients.

We hope these will satisfy the requirements by law and please contact me if you have any question regarding this matter. Thank you for your consideration and assistance.

Sincerely yours,

William W. Lee, Ph.D.
President

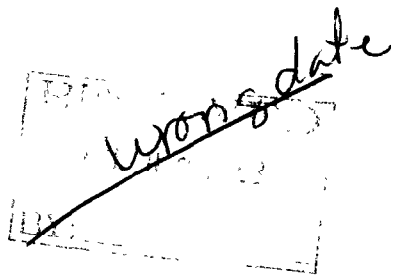
Enclosures

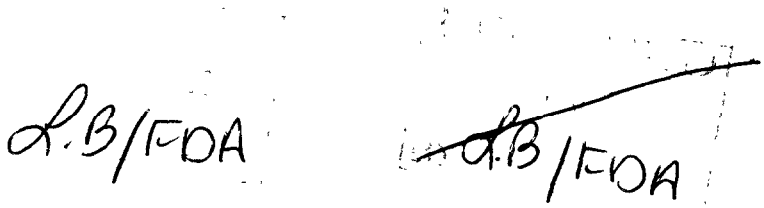
75-Day Premarket Notification for MuneGard

Volume 1

BioAge Pharmaceuticals, Inc.
9924 Mesa Rim Rd. #C3
San Diego, CA 92121 USA

75 Day Pre-marketing Notification for MuneGard™ Capsule





Submitted by: BioAge Pharmaceuticals, Inc.
9924 Mesa Rim Road
San Diego, CA 92121

Contact Info: William Lee, Ph.D.
BioAge Pharmaceuticals, Inc.
9924 Mesa Rim Road
San Diego, CA 92121
Phone 858-457-899
Fax 858-457-942
Email wwlee@bioagepharm.com

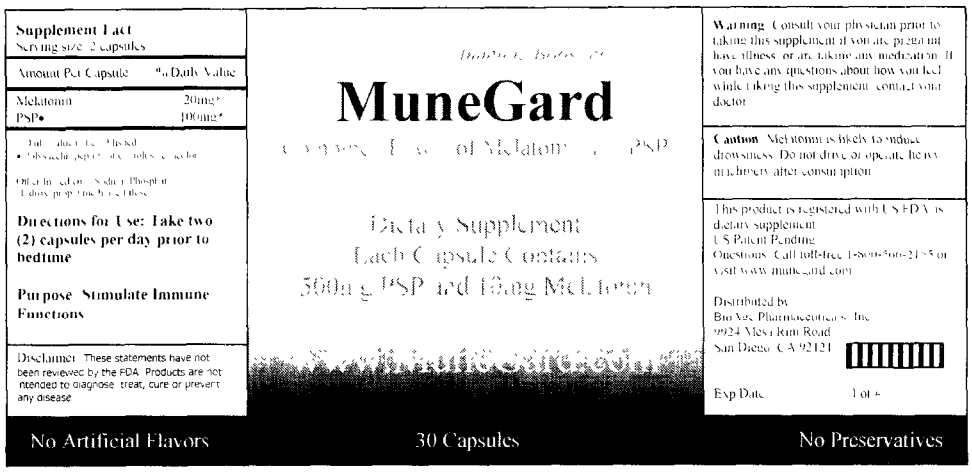
Table of Contents

1. Product Specifications

Product Name	MuneGard Capsule
Summary	MuneGard capsule is a trademarked dietary supplement with proteoglycan extract from *Coriolus versicolor* and melatonin as active ingredients, both of which have been reported to enhance immune functions and reduce toxic side effects from chemotherapy or radiation.
Distributor:	BioAge Pharmaceuticals, Inc. 9924 Mesa Rim Road, San Diego, CA 92121 USA
Content:	Each capsule contains 500mg proteoglycan extract of *Coriolus versicolor* and 10mg melatonin
Heavy Metals:	Lead <1.0ppm Arsenic <1.0ppm Mercury <0.5ppm
Microorganisms:	Standard Plate Count: <10 CFU/gram Salmonella: Not detected Yeast & Mold: <10CFU/gram
Packaging:	30 capsules per bottle Each capsule contains 500mg proteoglycan extract of *Coriolus versicolor* and 10mg melatonin
Instruction of use:	Take two (2) capsules per day prior to bedtime

2. Product Label

This dietary supplement is packaged in a 50ml vial and each vial contains 30 capsules. The label on the vial is as the following:



3. References

3.1 Safety Evaluation of Proteoglycan from *Coriolus versicolor*

Polysaccharide bound peptide or proteoglycan extracted from mushroom *Coriolus versicolor or Trametes versicolor* is an active ingredient of this dietary supplement.

Coriolus versicolor is a common mushroom in wooded area of Asia. North America and Europe. *Coriolus versicolor* is commonly referred to as Turkey Tail due to its morphological trait. In Chinese it is called Yun zhi, meaning cloud mushroom, and Kawaratake or mushroom by the riverbank in Japanese.

Coriolus versicolor grows on tree trunk and has long history in traditional Chinese medicine. Compendium of Materia Medica, the most complete encyclopedia of herbal medicine in China compiled in 1500s, listed Yun Zhi as herbal treatment to prolong life and invigorate health. Hot water extract is a common approach to prepare for herbal medicines in China and *Coriolus versicolor* has been used in this fashion for centuries.

Long history of folk remedy and anecdote reports of tumor fighting ability of *Coriolus versicolor* caught imagination of Japanese researchers in 1960s. After large scale screening, Japanese research identified and isolated polysaccharide kuresa (PSK) from CM-101 strain of *Coriolus versicolor* as active ingredient against tumor (1). Later in China, scientists isolated polysaccharide peptide (PSP) from Cov-1 strain of *Coriolus versicolor* (2).

Both PSK and PSP are proteoglycans or peptides attached to polysaccharides. Although there are reported minor differences between the two products, the composition, molecular weight, isolation process and effects of PSK and PSP are pretty much similar (1-3). For example, both PSK and PSP are produced with hot water extraction, a common process being practiced for traditional Chinese medicine and for Coriolus versicolor in particular. Their molecular weights are similar and chemical structures consist of peptide-bound polysaccharide. Most peptides are acidic amino acids and major linkages are β-1,3 or β-1,4 glucose linkage. They differ only in fucose, arabinose and rhamnose composition, all of which represent minor proportion of total carbohydrate. Thus, it is reasonable to assume that conclusions from studies can be generally regarded as applicable to both PSP and PSK.

PSK has been studied vigorously and over 350 articles have been published on this subject in peer-reviewed journals, including well-controlled clinical studies. PSK has been an approved drug in Japan since 1977 while PSP is used both as drug and dietary supplement in China.

Proteoglycans from *Coriolus versicolor* are remarkably safe in animal studies. Oral LD50 of PSP or PSK was reported to be over 20g/kg in mice and rat (3-4, 21). In monkeys, administration of 50 times of clinical dosage of PSP for 6 consecutive months produced no toxic reactions (3). Further more, oral doses up to 6g/kg in rats were administrated to study the effects of PSP on behavior and teratogenicity, the results showed no noticeable toxicity on fetus and animal behavior by PSP treatment (5). Ng &

Chan found that PSP may have analgesic activity but does not produce adverse effects on embryonic development in mice (6).

Proteoglycans from *Coriolus versicolor* has documented long-term human use. *Coriolus versicolor* has been used in traditional Chinese medicine for hundreds of years, as documented in Compendium of Materia Medica, and the common form used is hot water extract, a procedure similar to that of PSP or PSK production. In Japan, PSK treatment has been a common and popular cancer adjuvant treatment since its introduction in 1977. In China, PSP is available as approved medicine and as dietary supplement. After extensive clinical use for more than twenty years, there is no report to show toxic reaction or adverse drug interaction associated from using proteoglycan from *Coriolus versicolor*.

In the US, the dim prospect of gaining FDA approval for complex molecule and lack of strong intellectual protection rendered this agent an unlikely drug candidate for pharmaceutical companies regardless how effective it could be. However, the impressive safety profile made *Coriolus versicolor* or its extract an ideal choice as dietary supplement and indeed, *Coriolus versicolor* or its extracts are widely available as dietary supplement (7). JHS Natural Products introduced its version of proteoglycans from *Coriolus versicolor* as VPS since 1994. Currently, extract or raw species of *Coriolus versicolor* are widely available in the form of dietary supplement in the US.

There are many clinical studies that demonstrate complete safety of proteoglycans from *Coriolus versicolor*. Torisu et al. (8) conducted a double blinded, randomized and placebo controlled study in 111 colorectal cancer patients after curative surgery. Efficacy and side effects of PSK were investigated. PSK was taken orally at 3g per day for first two months after surgery, then 2g per day until twenty-four months and 1g per day thereafter. Patients were followed for eight years. Disease-free survival was significantly increased by PSK treatment. Side effects of PSK were also evaluated thoroughly. Besides

the increased incidents of coughing, which was due to powder formulation, nail pigmentation was the only significant side effect observed.

Nakazato et al. (9) studied PSK in combination with mitomycin and 5-fluorouracil in 262 gastric cancer patients. Patients were followed at least for 5 years and PSK was given orally at 3g per day with alternating every 4 weeks. Although the main objective of this study was to show the effectiveness of PSK, toxicities of all regimes were also studied in detail and the authors failed to identify any significant side effect attributed to PSK.

Another randomized trial was conducted in 73 acute non-lymphocytic leukemia patients with or without PSK in combination with chemotherapy. Patients were given oral PSK 3g per day and were followed for four years. Efficacy and side effects were evaluated. Again, no PSK related toxicities were observed (10).

Clinical studies of PSP have shown complete safety of this product. Large number of patients was administrated up to 6g PSP per day and no significant adverse reactions were noted (11).

There are also credible and respected institutions that offer information on herbal medicines and refer *Coriolus versicolor* as safe. For example, Natural Medicine Comprehensive Database collects broad and thorough data and reports on herbal medicines and lists *Coriolus versicolor* and its proteoglycans as possibly safe (12). Memorial Sloan-Kettering Cancer Center is a highly respected cancer caring facility. The website of Memorial Sloan-Kettering Cancer Center also offers comprehensive information for common herbs or botanic products. *Coriolus versicolor* and its proteoglycan extract was regarded safe and only possible adverse reaction was found to be darkening of toenails for some patients (13). MD Anderson Cancer Center is another highly respected cancer center and its website offers comprehensive and credible information about herbal medicines. *Coriolus versicolor* is regarded safe and side effects are cited as uncommon in the website (14).

In summary, *Coriolus versicolor* and its proteoglycan extracts have long history of human use in China and Japan. Clinical and animal studies show compelling evidence to support safety of *Coriolus versicolor* and its proteoglycan extracts. Most clinical studies use 3g PSP or PSK for several years without toxicity, thus, under the recommended dosage, this dietary component is reasonably expected to be safe.

3.2 Safety Evaluation of Melatonin

Melatonin is another major active ingredient in this dietary supplement. Melatonin is an endocrine-immune-modulating tryptophan derivative secreted from pineal gland. Melatonin has long been recognized as non-toxic and is one of the most popular dietary supplements available on the market today.

Melatonin was demonstrated to be highly safe in animal studies. Sugden (15) evaluated the psychopharmacological properties of melatonin in mice and rats. LD_{50} of oral melatonin was 1250mg/kg in mice and 3200mg/kg in rats. The recommended dose in this dietary supplement is 20mg per day or 0.33mg/kg, which is only one 3700^{th} of reported LD_{50} in mice.

There are many clinical studies to show that melatonin is safe. Lerner & Nordlund (16) reported clinical use of melatonin in 96 patients and daily doses up to 6.6g for 35 days did not produce general toxicity.

Seabra et al. (17) investigated the toxicity of melatonin in 40 healthy male volunteers. The trial was randomized and placebo controlled. Each volunteer took 10mg oral melatonin (n=30) or placebo (n=10) every day before sleep for 28 days. Biochemical parameters including blood glucose, lipids, proteins, and inorganic salts, as well as parameters of kidney, liver and hormonal functions were evaluated. No difference in any of these parameters was detected between melatonin and placebo treated groups, showing melatonin treatment was safe.

Voordouw et al. (18) studied the effects of melatonin on pituitary-ovarian axis in 32 birth-age women. Up to 300mg melatonin was administrated daily for four months. Hormonal, biochemical, hematological and physical parameters were evaluated in detail. The objective of this study was to investigate the hormonal suppression by melatonin, the authors did report that no differences in hematological and biochemical parameters were seen before, during or after melatonin treatment. This trial showed that melatonin could suppression hormonal levels (LH, E2 and P4) and might be used as an oral contraceptive. Other than these, administrated at 300mg daily for four months was safe and non-toxic.

There are many clinical studies to use melatonin to treat cancer patients as summarized in the functional aspect of this summary (25-32). These studies used melatonin at doses between 10-50mg daily for long period of time. Invariably, reduction of toxicity associated with chemotherapy or cancer was seen in melatonin treated group. There was no report of melatonin related toxicity. Although the purposes of these studies were to evaluate anti-tumor effects of melatonin, thorough biochemical, physiological and hematological evaluations were usually performed and are qualified to evaluate adverse reactions of melatonin. No melatonin related adverse reactions were reported. These studies show melatonin is safe for long-term use.

Melatonin does not affect genetic stability and does not cause mutations. Neville et al. (19) studied the mutagenicity of melatonin and its metabolite, 6-hydroxymelatonin. The authors demonstrated that neither melatonin nor 6-hydroxymelatonon is mutagenic in the presence or absence of an activation system in Ames test (23).

Taken together, animal and extensive clinical studies show that melatonin is safe at the dose recommended for this dietary supplement.

3.3 Functional References on Coriolus versicolor

Proteoglycans extracts of *Coriolus versicolor* exert their effect by modulating immuno functions, including activation of natural killer cells, T-cells and stimulation of

cytokine expression (1-2,20-21). The aim of this article is to summarize major clinical studies of proteoglycan extracts from *Coriolus versicolor* to demonstrate clinical safety.

Nakazato et al. (10) conducted a multi-center, randomized and controlled open label trial in 262 gastric cancer patients. After curative gastrectomy, patients were given chemotherapy consisted of intravenous mitomycin C on postoperative days 1 and 7 plus 150 mg/d oral fluorouracil. The PSK group received 3g/d oral PSK for 4 weeks alternating with 4 weeks fluorouracil, while control patients received only fluorouracil alternated with 4 weeks without treatment. Ten cycles were given to both groups. PSK patients experienced a greater 5-year disease-free rate (70.7% vs. 59.4%) and 5-year survival rate (73% vs 60%) than the control group. No adverse reactions associated with PSK treatment were observed.

Mitomi et al. (22) conducted a randomized, controlled multi-center study on adjuvant immunochemotherapy with PSK in curatively resected colorectal cancer. 448 patients received 6 mg/m2 MMC on the day of and day after surgery and 200 mg/day oral 5-FU for six months either with (n=221) or without (n=227) 3 g/day oral PSK for over 3 years. Demographics and clinical characteristics were similar between groups, except that PSK patients had significantly larger rectal tumors than controls. Patients were followed for 3-5 years. Both disease-free and overall survival curve were significantly better for patients receiving PSK ($p=0.013$): Three-year survival estimate for PSK patients was 85.8%, compared to 79.2% for controls.

Torisu et al. (9) tested PSK in a randomized, double blind evaluation of PSK (n=56) versus placebo (n=55) in patients after curative surgery of colorectal cancer. Starting 10-15 days after surgery, patients received 3 g/day PSK or placebo for 2 months, then 2 g/day until 24 months and 1 g/day thereafter. Clinical characteristics were similar between groups, although tumor size was not addressed. Overall and disease free survival were significantly higher in the PSK group compared to placebo ($p<0.05$, both). Polymorphonuclear monocytes from patients receiving PSK showed increased

phagocytic and locomotive activity. Other measured immune parameters, such as skin reactivity, lymphocyte counts, and immunoglobulin levels, did not differ significantly between groups.

Iino et al. (23) conducted a randomized evaluation of combination chemotherapy (5-fluorouracil, cyclophosphamide, mitomycin C, and predonisolone, FEMP) with 3 g/d PSK or 150mg/d levamisole (LMS) in 227 patients with operable breast cancer with vascular invasion in the tumor and/or in the metastatic lymph node. Each treatment, FEMP, FEMP+LMS, or FEMP+PSK, lasted 28 days and was carried out at 6-month intervals for 5 years. Patients receiving FEMP+PSK had a slightly better survival curve than the FEMP group (p=0.0706), although differences in survival and disease-free survival were not significant between the 3 groups. Ten-year disease free survival rates were 64.6% in the FEMP, 70.7% in the FEMP+LMS, and 74.1% in the FEMP+PSK group. No adverse reactions associated with PSK were reported.

Ohno et al. (11) conducted a randomized controlled trial of maintenance chemotherapy with or without PSK in 73 patients with acute nonlymphocytic leukemia (ANLL) who had achieved complete remission and had received consolidation therapy. Maintenance chemotherapy was given alternately every 5th week for 2 years. 3 g/d PSK was given every day indefinitely except during maintenance chemotherapy. Remission duration and survival length analyzed 6 months after the last entry showed borderline beneficial effect of PSK (p=0.089), but analysis at 24 months showed no significant difference.

These clinical trials were generally well designed and conducted for a long period of time. Clear benefits of proteoglycan from *Coriolus Versicolor*, including survival extension and reduction of toxicity induced by chemotherapy were seen for gastric and colorectal cancer patients.

3.4 Functional References on Melatonin

Melatonin is an important regulator of immunoneuroendocrine system and exerts diverse functions (24). The aim for this dietary supplement is to focus on reduction of toxic side effects of cancer chemo- or radiotherapy. We thus incorporated clinical studies relevant with cancer treatment to show that melatonin at recommended dose of this dietary supplement has been safely used in cancer patients for extended time.

Melatonin treatment alone showed clear benefits in patients with non-treatable solid tumors. For example, Lissoni et al. (25) reported a randomized study with melatonin versus supportive care in advanced non-small cell lung cancer patients. Thirty-one patients received 10mg melatonin per day orally at 7:00pm and thirty-two received supportive care alone including non-steroid anti-inflammation drugs and opioids for pain. Both groups had comparable parameters initially. After one year, survival and stable disease rates were both significantly higher in melatonin treated group than supportive care group. There was no melatonin associated adverse reactions, on the contrary, significant improvement in performance status was seen in melatonin treated patients.

A similar study to investigate the effectiveness of melatonin was conducted in 30 node-relapsed melanoma patients (27). Patients received either supportive care (n=16) or 20mg melatonin (n=14) daily before sleep till disease progression. Both groups had comparable characteristics at the beginning. Minimal and median follow up were one year and 31 months, respectively. At one year after treatment, disease-free survival was significantly higher in melatonin treated group (10/14 vs 5/16, $p<0.05$). After median follow up or 31 month, relapse occurrence was significantly less in melatonin treated group (4/16 vs 11/16, $p<0.05$). There was no toxicity associated with melatonin treatment and relief of anxiety was experienced by most patients received melatonin.

The effect of melatonin was further confirmed in a large trial consisting of 1440 patients with untreatable advanced solid tumors (27). Patients received either supportive care plus 20mg oral melatonin (n=722) daily in dark period or supportive care alone

(n=718), which included non-steroid anti-inflammation drugs and opioids. After one year, the percentage of patients with objective response and with stable disease as well as survival was significantly higher in melatonin treated group. No adverse reactions associated with melatonin treatment were observed. On the contrary, significant improvements in cachexia, asthenia, anorexia, depressive symptoms, thrombocytopenia and lymphocytopenia were seen in melatonin treated group than support care alone.

The improvement of performance status particularly cachexia in melatonin treated cancer patients was studied in a randomized controlled study in 86 patients with untreatable solid tumors (28). Patients received either supportive care, including non-steroid anti-inflammatory agents and pain treatment (n=41), or supportive care plus 20mg oral melatonin in the evening (n=45). Three months after treatment, there was no melatonin-related toxicity observed and most patients treated with melatonin experienced relief of asthenia and improvement in performance status. The number of patients experienced more than 10% body weight loss was significantly less in melatonin treated group (4% vs 32%, $p<0.01$). The mean weight loss was significantly lower in melatonin treated group at 1, 2, and 3 month after treatment ($p<0.05$, $p<0.05$, $p<0.01$). Percentage of disease progression was significantly lower in melatonin treated group (53% vs 90%, $p<0.05$).

The above studies were conducted in cancer patients with untreatable solid tumors and very poor performance status. These clinical trials clearly demonstrate that melatonin can be safely administrated to these very sick patients and no melatonin-related toxicity was observed. On the contrary, melatonin treatment invariably improved performance status and quality of life.

Immunotherapy is an important aspect of cancer treatment and since melatonin is immuno-stimulatory, clinical trials have been conducted to investigate combination therapy with melatonin plus IL-2, a classic immunotherapy agent.

Lissoni et al. (29) conducted a randomized and controlled trial to investigate the effect of melatonin in combination with IL-2. Eighty patients with non-renal and non-melanoma solid tumors were treated with IL-2 alone (n=39) or IL-2 plus melatonin (n=41). Both groups had comparable characteristics. After one year, patient in melatonin/IL-2 group showed significantly higher response rate (complete & partial response) that those treated with IL-2 only (26% vs 3%, $p<0.001$). Moreover, the percentage survival at one year was also significantly higher in IL-2/melatonin group (19/41 vs 6/39, $p<0.05$). When side effects were examined, IL-2/melatonin groups showed better performance than IL-2 alone.

Chemotherapy is a major weapon against malignancies but can induce severe toxicities, including bone marrow suppression, cachexia, alopecia, mucositis, nephrotoxiciy, neurotoxicity and more.

Clinical studies demonstrate that melatonin treatment in combination with chemotherapy reduce toxic side effects. Lissoni et al. (30) conducted a randomized controlled study in 70 advanced non-small cell lung cancer patients to evaluate effects of chemotherapy or chemotherapy plus melatonin. Patients received either cisplatin at 20mg/m2/day iv for 3 days and etoposide at 100mg/m2/day iv for 3 days (n=36) or chemotherapy plus melatonin at 20m/day orally in the evening. The percent of one-year survival was significantly greater in patients receiving melatonin than in those treated with chemotherapy alone (15/34 vs 7/36, $p<0.05$). Moreover, the survival curve was significantly longer in patients concomitantly treated with melatonin ($p<0.05$). Chemotherapy related toxicity was less pronounced in patients receiving melatonin. In particular, melatonin related improvements were significant in myelosuppression, neuropathy, asthenia and weight loss.

Lissoni et al. (32) conducted a randomized trial in 250 patients with metastatic advanced solid tumors. Various chemotherapy agents were used for different types of tumor (cisplatin+etoposide for 40, and gemcitabine for 12 NSCLC patients; doxorubicin

for 19, mitoxantrone for 13 and paclitaxel for 6 breast cancer patients; 5-FU+folinic acid for 22 GI tract cancer patients; and 5-FU+cistplatin for 14 head and neck cancer patients, total patient n=126). For each chemotherapy regime, matched number of patients was treated with chemotherapy plus melatonin (n=124). After one year, overall response rate (complete plus partial response) was significantly increased (15% vs 34%, $p<0.001$) for group with melatonin plus chemotherapy. The one-year survival rates for each chemotherapy regime except mitoxantrone were all significantly improved by addition of melatonin. Overall one-year survival was also significantly better in melatonin added group (23% vs 51%, $p<0.001$). Among the chemotherapy toxicities, significant improvements were observed in myelosuppression, thrombocytopenia, neurotoxicity, nephrotoxicity, cardiotoxicity, stomatitis, and asthenia after melatonin treatment.

A similar study as previous one was also reported (27). Two hundred patients with advanced solid tumors were received chemotherapy (43 NSCLC patients, 18 treated with cisplatin/etoposide, 11 treated with cisplatin/taxol, 14 treated with cisplatin/gemcitabine; 26 colorectal cancer patients, 14 treated with 5-FU/folates, 12 treated with paclitaxel; 11 gastric cancer treated with cisplatin/epirubicin/5-FU/folates; and 22 soft tissue sarcomas, 12 treated with adriamycin/ifosfamide, 10 treated with ifosfamide; total patients n=102) or chemotherapy plus 20mg/day oral melatonin given in dark period (n=98). After one year, the objective response rate was significantly higher in melatonin treated group than chemotherapy alone (30% vs 20%, $p<0.05$). The concomitant administration of melatonin significantly reduced the percentage of patients with asthenia, thrombocytopenia, neurotoxicity, cardiotoxicity, and stomatitis. One-year survival curve in patients concomitantly treated with melatonin was significantly higher than that for patients received chemotherapy alone ($p<0.05$).

The toxic effects of radiation therapy are similar to those of chemotherapy and may be also antagonized by melatonin treatment. Lissoni et al. (32) conducted a randomized controlled trial in 30 gliobastoma patients. Patients received either radiotherapy (n=16) or radiotherapy plus 20mg/day melatonin orally in dark period. The percent of survival at

one year was significantly higher in patients treated with melatonin plus radiotherapy (6/14 vs 1/16, $p<0.02$) and the survival curve was significantly longer in melatonin treated group ($p<0.05$). No melatonin related toxicity, particularly no clinically evident interference between melatonin and anti-convulsants, was observed. In contrast, most patients receiving melatonin experienced a relief of anxiety and improvement of quality of sleep.

These clinical studies clearly demonstrate the beneficial effects of melatonin alone or in combination with immunotherapy, chemotherapy or radiotherapy. No melatonin related toxicities are observed. On the contrary, improvements in quality of life or reduction of toxicity from other modalities are invariably seen. More importantly, melatonin treatment at recommended dose (20mg per day) is safe in these very sick patients and reduces side effects induced by other modalities.

4. Conclusions

Extensive clinical studies show that melatonin and proteoglycans from *Coriolus versicolor* have long and safe history of human use. Clinical evaluations show that both melatonin and proteoglycan of *Coriolus versicolor* can be reasonably expect to be safe under the recommended usage in this dietary supplement.